

Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Initially, applicants would like to note that the present amendment is being submitted in compliance with "Amendments In A Revised Format Now Permitted", 1267 OG 4 (February 25, 2003). Pursuant to this notice, the requirements of 37 C.F.R. § 1.121 have been waived.

Applicants respectfully request reconsideration of the restriction as between the proteins of SEQ ID NO: 7 and SEQ ID NO: 66. While applicants do not dispute that these two proteins have different structure due to their different amino acid sequences, applicants submit that the structures are quite similar as evidenced by their 78% amino acid identity (see Exhibit attached to response submitted on December 4, 2002). Because the two proteins are quite similar and it is therefore expected that they have a similar (if not the same) function, rather than a different function as asserted by the U.S. Patent and Trademark Office ("PTO"). As further evidence of their similar structure and function, attached hereto is Badel et al., "A Gene in the *Pseudomonas syringae* pv. *tomato* Hrp Pathogenicity Island Conserved Effector Locus, *hopPtoA1*, Contributes to Efficient Formation of Bacterial Colonies in Planta and Is Duplicated Elsewhere in the Genome," MPMI 15(10):1014-1024 (2002) ("Badel") (copy attached as Exhibit A). Badel reports that HopPtoA (of SEQ ID NO: 7) and HopPtoA2 (of SEQ ID NO: 66) are highly similar: HopPtoA is a basic, alanine-rich (17.1%) 486-residue protein of 50.7 kDa with a pI of 9.06 and HopPtoA2 is a basic, alanine-rich (15.0%) 487-residue protein of 51.1 kDa with a pI of 9.16 (see Badel at page 1016, second column); both HopPtoA and HopPtoA2 are secreted in a hrp-dependent manner (see Badel at 1017, first column); and based on mutation studies, *hopPtoA* and *hopPtoA2* contribute redundantly to the formation of *P. syringae* pv. *tomato* DC3000 colonies in *Arabidopsis* leaves (see Badel at 1017-1018 and abstract). Thus, the proteins of SEQ ID NOS: 7 and 66 are both structurally and functionally similar. For these reasons, the inventions are clearly related and, given that the search burden is minimal, the proteins should be examined together as presently claimed in the existing Markush group. Applicants respectfully request withdrawal of the restriction as between the presently claimed subject matter.

The objections to claims 7-9 are respectfully traversed in view of the above amendments.

The rejection of claims 7 and 8 under 35 U.S.C. § 102(b) as anticipated by Charkowski et al., "HopPtoA, a *Pseudomonas syringae* pv. *tomato* Hrp-secreted Protein with Homology to Pectate Lyases," Phytopathol. 87(6):S17 (1997) ("Charkowski I") is respectfully traversed.

Charkowski I describes the isolation of a 1,272 bp gene identified as *hopPtoA* and describes various properties of the predicted protein, HopPtoA. These properties include the presence of a region that contains eight imperfect glycine-rich repeats and a C-terminal region homologous to pectate lyases. To support its position that Charkowski I describes the isolation of HopPtoA, the PTO also cites to Charkowski et al., "The *Pseudomonas syringae* pv. *tomato* HrpW Protein Has Domains Similar to Harpins and Pectate Lyases and Can Elicit the Plant Hypersensitive Response and Bind to Pectate," J. Bacteriol. 180(19):5211-5217 (1998) ("Charkowski II").

Despite its original designation as HopPtoA, the protein described in Charkowski I is now known as HrpW. This fact is supported by Charkowski II, which recites:

HrpW was found to be identical to the previously identified and partially sequenced transcriptional unit V and to encode the previously identified Hrp-secreted protein EXP-60 (Lorang and Keen, "Characterization of *avrE* from *Pseudomonas syringae* pv. *tomato*: A hrp-linked Avirulence Locus Consisting of at Least Two Transcriptional Units," Mol. Plant Microbe Interact. 8:49-57 (1995); Yuan and He, "The Hrp Regulation and Secretion System Controls the Production and Secretion of Multiple Extracellular Proteins," J. Bacteriol. 178:6399-6402 (1996)). **In a preliminary report, this protein was referred to as HopPtoA (Charkowski et al., "HopPtoA, a *Pseudomonas syringae* pv. *tomato* Hrp-secreted Protein with Homology to Pectate Lyases," Phytopathol. 87(6):S17 (1997)).** It is now designated HrpW based on its HR elicitor activity, the phenotype of a *hrpZ hrpW* mutant, and the homology of the protein with the HrpW proteins from *E. amylovora* strains Ea321 (Kim and Beer, "HrpW of *Erwinia amylovora*, a New Harpin That Contains A Domain Homologous to Pectate Lyases of a Distinct Class," J. Bacteriol. 180:5203-5210 (1998)) and CFBP1430 (Gaudriault et al., "HrpW of *Erwinia amylovora*, a New Hrp-secreted Protein," FEBS Lett. 428:224-228 (1998)).

Charkowski II at 5215, first and second columns (emphasis introduced). The above passage clearly demonstrates that the protein preliminarily designated HopPtoA by Charkowski I is the same protein designated HrpW by Charkowski II. Therefore, contrary to the PTO's

assertion at page 4 of the outstanding office action, Charkowski II does *not* disclose that the protein described in Charkowski I has the identical sequence of SEQ ID NO: 7.

A comparison of the protein of Charkowski I and II (HrpW) with the protein of SEQ ID NO: 7 demonstrates that these proteins are *not* the same. Specifically, HopPtoA (SEQ ID NO: 7) contains 486 amino acid residues, while HrpW of Charkowski I and II possesses 424 amino acid residues. In addition, both Charkowski I and II identify the presence of glycine rich repeats in HrpW (see Figure 1C of Charkowski II), whereas HopPtoA possesses no such glycine rich repeats (see SEQ ID NO: 7). A ClustalW alignment, performed using default parameters (<http://www.embl-heidelberg.de>) with the *Pseudomonas syringae* pv. *tomato* DC3000 HrpW amino acid sequence of GenBank Accession AF005221 and the *Pseudomonas syringae* pv. *tomato* DC3000 HopPtoA amino acid sequence of SEQ ID NO: 7, confirms that the two proteins are not the same (see Exhibit B attached hereto).

Moreover, a GeneStream alignment, performed using default parameters (<http://xylian.igh.cnrs.fr>) with the *Pseudomonas syringae* pv. *tomato* DC3000 *hrpW* nucleotide sequence of GenBank Accession AF005221 and the *Pseudomonas syringae* pv. *tomato* DC3000 *hopPtoA* nucleotide sequence of SEQ ID NO: 6, confirms that these two nucleic acid molecules are only about 48 percent identical (see Exhibit C attached hereto). There exist numerous gaps along the length of the alignment shown in Exhibit C and the two sequences contain no regions of high similarity that possess more than eight consecutively aligned bases. Therefore, applicants submit that the complement of the *hrpW* nucleic acid molecule would *not* hybridize to the nucleotide sequence of SEQ ID NO: 6 under the conditions recited in claim 1.

From the foregoing, it should be apparent that Charkowski I does not teach or suggest the isolated protein of SEQ ID NO: 7 let alone an isolated protein as recited in claim 7. Therefore, the rejection of claims 7 and 8 as anticipated by Charkowski I is improper and should be withdrawn.

The rejection of claims 7-9 under 35 U.S.C. § 103 for obviousness over Yuan et al., "The *Pseudomonas syringae* Hrp Regulation and Secretion System Controls the Production and Secretion of Multiple Extracellular Proteins," *J. Bacteriol.* 178(21):6399-6402 (1996) ("Yuan") in view of Charkowski I is respectfully traversed.

Yuan identifies seven proteins that are under the production and secretion control of the Hrp regulatory system in *Pseudomonas syringae* pv. *tomato* DC3000, one of which is designated EXP-60.

Charkowski I is cited substantially as described above.

The PTO cites to Yuan as identifying the isolated protein EXP-60, which the PTO suggests is the same as HopPtoA of the presently claimed invention (citing Charkowski I for support). EXP-60, however, was later designated HrpW (see Charkowski II at 5215 (text quoted above)). Because EXP-60 is the same protein as HrpW, which for the reasons noted above is distinct of HopPtoA (SEQ ID NO: 7) and the isolated protein as presently claimed, applicants submit that Yuan and Charkowski I, either alone or in combination, fail to teach or suggest the presently claimed protein.

For these reasons, the rejection of claims 7-9 for obviousness over Yuan in view of Charkowski I is improper and should be withdrawn.

Applicants note that a supplemental information disclosure statement was submitted on February 13, 2003, prior to receipt of the outstanding office action by applicants undersigned attorney. Applicants respectfully request consideration of the references cited thereon and the return of the signed form PTO-1449 with the next office action. If a fee is required for consideration of the references listed in the supplemental information disclosure statement, then the undersigned attorney hereby authorizes the PTO to charge deposit acct. 14-1138 in the amount of \$180.00.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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Date <u>4/17/03</u>	<u>Wendy L. Barry</u>